

## Chapter 2

# Cannabinoids Modulation of Emotional and Non-Emotional Memory Processes After Stress

Irit Akirav

**Abstract** The endocannabinoid system (ECS) is involved in regulating the stress response and subsequent changes in neuroendocrine function and emotional behavior. It is also a critical neuromodulatory system that affects learning and memory. Generally systemically administered cannabinoid agonists have an impairing effect on memory processes although enhancing effects are also reported.

Stress is a potent modulator of brain function and cognition that has differential effects on memory function depending on a number of factors (such as stress duration, stress intensity, timing and the source of the stress, as well as the learning type under study). Most of the tasks to investigate learning and memory in laboratory rodents are stressful for the animals (i.e. the cognitive task includes intrinsic stress) as opposed to extrinsic stress which refers to outside stress that occurs before or after the cognitive task. Several lines of evidence suggest that cannabinoids differentially affect different memory phases (acquisition, consolidation, retrieval and extinction), and that the type of cognitive task (emotional or aversive versus non-emotional) also determines the neural substrates underlying the effects of cannabinoids on memory.

In this chapter I will describe the interaction between the effects of activating the ECS and stress exposure on emotional (i.e., aversive) and non-emotional learning and memory processes in animal models. I will argue that administering cannabinoid agonists in proximity to extrinsic stress exposure normalizes stress modulation of emotional memory. A possible model of the effects of cannabinoids on emotional memory after stress is also presented.

**Keywords** Endocannabinoids · Learning and memory · Emotional learning · Stress · Hypothalamic-pituitary-adrenal axis

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## Introduction

The endocannabinoid system (ECS) plays a modulatory role in many cognitive and emotional processes. Cannabis has been used recreationally for its mood-enhancing and stress-alleviating properties for centuries. The discovery of  $\Delta 9$ -tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana, along with its biologically active analogs has stimulated extensive research devoted to understanding how these compounds exert their effects on emotionality within the brain.

Multiple animal models have been used to assess the effects of the ECS on various stages of memory (acquisition, consolidation, retrieval and extinction), using a wide range of behavioral paradigms [1–5]. Both acute and chronic exposure to cannabis is associated with impairments in attention, working memory, verbal learning and memory functions [5–8]. Working memory refers to a brain system that provides temporary storage and manipulation of the information necessary for complex cognitive tasks. Long-term heavy cannabis users show impairments in memory and attention that, depending on the task analyzed, might be reversible [9], although in some cases they persist beyond the period of intoxication and get worse with increasing years of regular cannabis use [10].

Most of the studies that examined the effects of the ECS on hippocampal-dependent memory focused on spatial learning [3]. Spatial learning is the acquisition of information about one's surroundings. In general, the findings are that exogenous and endogenous cannabinoid agonists impaired working memory and the acquisition of long-term memory [11], but had no effect on memory retrieval [12–13]. Other studies, usually focused on fear-related paradigms, demonstrated that activating the ECS facilitated extinction [4, 14–15]. Hence cannabinoids differentially affect the different memory phases but it seems that the type of cognitive task (emotional or aversive versus non-aversive) also determines the neural substrates underlying the effects of cannabinoids on memory [5]. The effects of inhibiting the ECS on learning and memory has been reviewed elsewhere [16].

Most of the tasks currently used to investigate learning and memory in laboratory rodents can be considered as being stressful for the animals: they are based on the application of stressful manipulations and/or stimuli to motivate animals to learn. “Intrinsic stress”, refers to situations in which stress is originated by elements related to the cognitive task, and “extrinsic stress”, refers to those situations in which stress is originated by conditions completely unrelated to the cognitive task and thus generally occurring temporally dissociated from such task (i.e., either before or afterwards) [17].

Intrinsic stress that is related to the cognitive task generally enhances the consolidation of memory through actions of norepinephrine and glucocorticoids on the neural circuits activated by the learning experience (see review by [17]). When the stress derives from conditions other than the cognitive task (i.e. extrinsic stress), then the effects are more varied and more specific to the type of learning involved [17]. For example, acute extrinsic stress enhanced aversive hippocampal-dependent tasks such as contextual fear conditioning and trace eye-blink conditioning

(e.g., [18–19]), but impaired hippocampal-dependent spatial memory retrieval (see review by [20]). In contextual fear conditioning an animal learns the association between the shock (i.e., the unconditioned stimulus) and the context in which conditioning occurs (the conditioned stimulus) whereas in trace eye-blink conditioning the animal learns the association between a shock or an air puff (i.e., the unconditioned stimulus) and a tone (the conditioned stimulus).

Stress is known to be a potent modulator of brain function and cognition. While prolonged and/or excessive stress generally exerts negative effects on learning and memory processes, acute stress can have differential effects on memory function depending on a number of factors (such as stress duration, stress intensity, timing and the source of the stress, as well as the learning type under study).

In this chapter I will describe the modulatory effects of activating the ECS on aversive (i.e., emotional) and non-aversive learning and memory processes in animal models, with or without exposure to extrinsic environmental stress. I will argue that activating the ECS has a different effect on learning and memory processes when ECS activation occurs shortly before or after an exposure to a stressful experience. Hence, the administration of cannabinoid agonists or exposure to stress may enhance or impair memory, but when cannabinoid agonists are administered in proximity to stress exposure (i.e., before or after stress exposure), they prevent the stress-induced alterations in memory. To summarize I present a possible model of the effects of cannabinoids on memory after stress that involves the interaction between the ECS and the hypothalamic-pituitary-adrenal axis system.

## The Endocannabinoid System and Emotional Memory

The ECB system, which includes cannabinoid receptors (CB1 and CB2) and endocannabinoids (*N*-arachidonylethanolamine [anandamide; AEA] and 2-arachidonoyl-glycerol [2-AG]) [21–23], has been repeatedly implicated in the effects on emotionality within the brain. The ECS has recently emerged as a promising therapeutic target for the treatment of stress-related emotional disorders [24–27]. In support, a growing literature base has collectively demonstrated that facilitation of endocannabinoid signaling promotes antidepressant- and anxiolytic-like responses in pre-clinical animal models [15, 28–33].

Emotional learning is extremely important for the survival of an individual. In studies of emotional behavior, the amygdala, medial prefrontal cortex, and hippocampus have received the most attention because structural and functional abnormalities within these regions are most commonly associated with mood disorders in clinical populations [34]. These three areas are a key circuit in the adaptive and maladaptive responses to stress as they undergo stress-induced structural remodeling, which alters behavioral and physiological responses, including anxiety, aggression, mental flexibility, memory and other cognitive processes [35, 36]. This is in accordance with the fact that both glucocorticoid receptors and CB1 receptors are located within this brain circuit [37–40].

The amygdala acts as an interface between sensory inputs and cortical processing, and activation of this structure is directly linked to the generation of fear and anxiety [41–42], and promotes activation of the hypothalamic-pituitary-adrenal axis [43]. The prefrontal cortex is involved in higher-order processing and is explicitly involved in the recognition of aversive stimuli and in drawing conclusions about the controllability of stimuli [44–45]. The hippocampus interacts with the prefrontal cortex to suppress the hypothalamic-pituitary-adrenal axis and promote recovery to homeostasis following a stressful experience [46].

### ***The Effects of Cannabinoids on Aversive and Non-Aversive Learning Tasks***

There have been reports of deficits in memory following administration of cannabis extracts or cannabinoid agonists in rodents and in humans and this has been reviewed extensively before [47–55]. Nevertheless, several reports suggest enhancing effects of cannabinoids on memory [56–57].

I will briefly describe some of these studies that together demonstrate apparent discrepancies in the effects of cannabinoid agonists on learning and memory. Several reasons could explain these effects, among them are different spatial and temporal ways of activating cannabinoid receptors by exogenous pharmacological agents; the time point of drug administration in relation to the cognitive task being examined; systemic versus local intra-cerebral administration of the drugs; the different pharmacokinetics of each drug that can affect the system for hours or days. Other reasons are related to the memory phase under investigation, the aversiveness of the cognitive task etc.

#### **Non-Aversive Memory Tasks**

As described earlier, most of the memory tasks in animals involve an aversive component. In recognition tasks, on the other hand, no rewarding or aversive stimulation is used during training, so the learning occurs under conditions of relatively low stress or arousal [58]. Object recognition memory is the ability to discriminate the familiarity of previously encountered objects and it is based on the spontaneous exploration behavior of the rat.

Systemic administration of  $\Delta^9$ -THC or the CB1/2 receptor agonist WIN55,212–2 impaired object recognition in rats [59–60]. Suenaga & Ichitani [61] found that intra-hippocampal WIN55,212–2 (1–2  $\mu$ g/site) did not affect object recognition memory but impaired the ability to recognize an object that was moved to another location (hippocampal-dependent spatial recognition).

Recently, Campolongo et al. [62] investigated the effects of cannabinoid administration on both short- and long-term object recognition memories under two experimental conditions that differed with respect to their training-associated arousal level (i.e., by manipulating the level of habituation to the apparatus). They found

**Table 2.1** The effects of cannabinoids on performance in non-aversive tasks

Species	Drug	Test	Effect on memory	References
Rat	$\Delta^9$ -THC 5 mg/kg, IP	Object recognition	Impaired acquisition	[59]
Rat	WIN55,212–2 1.2 mg/kg, IP	Object recognition	Impaired acquisition	[60]
Rat	WIN55,212–2 1–2 $\mu$ g, intra-hippocampal	Object recognition	No effect	[61]
Rat	WIN55,212–2 0.3 mg/kg, IP	Object recogni- tion-habituated	No effect retrieval	[62]
Rat	WIN55,212–2 1–2 $\mu$ g, intra-hippocampal	Spatial recognition	Impaired acquisition	[61]
Rat	WIN55,212–2 0.6 or 1.2 mg/kg, IP	Object recognition	Impaired acquisition	[63]
Rat	WIN55,212–2 0.6 or 1.2 mg/kg, IP	Social recognition	Impaired acquisition	[63]
Rat	WIN55,212–2 5 $\mu$ g, intra-hippocampal	Social recognition	Impaired acquisi- tion and retrieval	[64]
Rat	WIN55,212–2 5 $\mu$ g, intra-BLA	Social recognition	Impaired retrieval	[64]

A general summary of the pharmacological studies examining the effects of exogenous cannabinoids on non-aversive memory paradigms.  $\Delta^9$ -THC,  $\Delta^9$ - tetrahydrocannabinol, *IP* Intraperitoneal

differential effects of cannabinoids depending on the rats’ level of arousal and the memory under investigation (short- versus long-term memory). Post-training systemic administration of WIN55,212–2 (0.3 mg/kg) impaired short-term retention while enhancing long-term retention in non-habituated highly aroused rats. In habituated rats, WIN55,212–2 enhanced short-term retention with no effect on long-term retention.

The social recognition test is similar to the object recognition test but uses conspecifics instead of objects as the stimuli. WIN55,212–2 (0.6 and 1.2 mg/kg) impaired the performance of rats in the social and object recognition task [63]. We found that WIN55,212–2 (5  $\mu$ g/side) impaired acquisition and retrieval of social recognition when microinjected into the hippocampus and impaired acquisition when microinjected into the medial amygdala [64]. See Table 2.1 for a general summary of the pharmacological studies examining the effects of exogenous cannabinoids on non-aversive memory paradigms.

**Aversive Memory Tasks**

Water maze procedures which focus on spatial memory have been extensively used to test the effects of cannabinoids on different stages of hippocampal-dependent

memory. In the water maze task, the animals are trained to escape to a submerged platform in a tank filled with opaque water suggesting that this task is aversive.

In mice and rats, acute systemic administration of  $\Delta 9$ -THC [8 mg/kg, Intraperitoneal (IP)] or WIN55,212-2 (1 and 3 mg/kg) before the training session disrupted acquisition in the water maze test [65–66]. However,  $\Delta 9$ -THC in doses known to impair acquisition did not impair memory retrieval in the water maze [67–68]. Impaired place-learning in the water maze was also demonstrated in rats treated repeatedly with  $\Delta 9$ -THC [69] or acutely with  $\Delta 8$ -THC [70] or synthetic CB1 receptor agonists such as HU-210 [50], but not with the synthetic agonist nabilone [70].

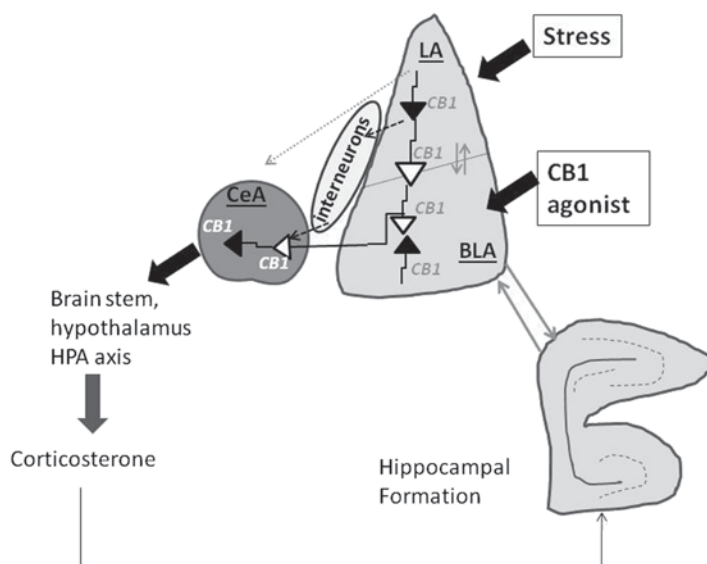
In contextual fear conditioning, the agonist WIN55,212-2 (2.5 and 5.0 mg/kg), given 30 min before the conditioning phase, impaired acquisition of contextual fear conditioning [54]. When WIN55,212-2 (10 or 30 ng in 0.5  $\mu$ L) was infused into the hippocampus 1 h before the retention test, it impaired retrieval of contextual fear memory [71].

In the inhibitory (passive) avoidance task, systemic injections of  $\Delta 9$ -THC or intra-hippocampal injections of WIN55,212-2 impaired memory acquisition, consolidation, and recall in rats and mice [67, 72]. We found that intra-basolateral amygdala or intra-CA1 WIN55,212-2 (5  $\mu$ g) had no effect on inhibitory avoidance conditioning [14, 73]. In the light-dark inhibitory avoidance paradigm the animal experiences a pairing of a previously neutral stimulus, the dark context, with an aversive stimulus, footshock, a pairing which results in an increase in latency to enter the dark chamber at testing. Interestingly, several studies found that cannabinoid agonists may enhance memory consolidation. Intra-basolateral amygdala WIN55,212-2 (5–50 ng per side), infused immediately after inhibitory avoidance training, induced dose-dependent enhancement of 48-h retention [56] and propofol, which inhibits fatty acid amide hydrolase, the enzyme that degrades the endocannabinoid anandamide, administered intraperitoneally after training also significantly increased memory consolidation [57]. See Table 2.2 for a general summary of the pharmacological studies examining the effects of exogenous cannabinoids on aversive memory paradigms.

Taken together, these results suggest that the type of cognitive task can determine the neural substrates underlying the memory impairment produced by cannabinoids. The time of drug injection in relation to the learning phase under examination is also a critical factor. Finally, it should be noted that the fact that cannabinoid receptors are localized in different brain structures suggests the modulation of distinct memory process and may explain cases where microinfusion of cannabinoid compounds into specific areas can produce effects different from those seen with systemic administration.

## Extinction

Fear inhibition in the form of extinction learning is also considered as an aversive or at least an emotional learning paradigm. In the majority of the studies described,



**Fig. 2.2** Intra-basolateral amygdala CB1 receptor agonist immediately after stress exposure and hypothalamic-pituitary-adrenal axis activation reduces the stress response via GABAergic mechanism. The lateral amygdala (LA) is connected to basolateral amygdala (BLA) and central amygdala (CeA). A sub-population of LA neurons innervates inhibitory interneurons, which in turn are connected to CeA by inhibitory synapses. The CeA represents a main output station of the amygdala to the brain stem and hypothalamus (and the hypothalamic-pituitary-adrenal axis). A most dominant distribution of CB1 receptors is found in GABAergic (full arrow) and glutamatergic (empty arrow) neurons in the BLA and CeA. Intra-BLA CB1 receptor agonist administered immediately after stress exposure reduces GABA release in BLA interneurons, thereby reducing their inhibition of the GABAergic neurons of the intercalated nuclei, which, in turn, increases their inhibition of the pyramidal neurons of the CeA. Hence, CB1 receptor agonists can reduce hypothalamic-pituitary-adrenal axis activation (and corticosterone release) and modulate the effects of stress on emotional memory. Hence, cannabinoid receptor activation after stress exposure prevents the stress-induced increase in corticosterone levels. The BLA is reciprocally connected with the hippocampal formation. Hence, the amygdala may modulate hippocampal-dependent memory processes directly or indirectly via its effects on the hypothalamic-pituitary-adrenal axis (e.g. as corticosterone readily enters the brain and binds to glucocorticoid receptors in the hippocampus to affect memory). (Data was published by [95] in *Neurosci Biobehav Rev*)

a reduction in the stress-induced increase in corticosterone levels. Corticosterone easily re-enters the brain to affect glucocorticoid receptors in brain areas that are highly involved in memory processes (e.g. the hippocampal formation). Hence, the reduction in hypothalamic-pituitary-adrenal axis activity may prevent the enhancing or the impairing effects of stress on emotional memory. In support, it has been shown that CB1 receptor agonists decrease the excitability of projection neurons in the rat basolateral amygdala [108]. Several studies have shown that activating CB1 receptors or increasing AEA signaling, prevents some of the effects of stress in the

**Table 2.2** The effects of cannabinoids on performance in aversive tasks

Species	Drug	Test	Effect on memory	References
Mice	$\Delta$ 9-THC 6 or 10 mg/kg, IP	Water maze	Impaired acquisition	[65]
Rat	WIN55,212-2 1 or 3 mg/kg, IP	Water maze	Impaired acquisition	[66]
Rat	$\Delta$ 9-THC 6 or 10 mg/kg, IP	Water maze	No effect retrieval	[67]
Mice	$\Delta$ 9-THC 3, 10 or 30 mg/kg, IP	Water maze	No effect retrieval	[68]
Rat	WIN55,212-2 2.5 or 5 mg/kg, IP	Contextual fear conditioning	Impaired acquisition	[54]
Rat	WIN55,212-2 10 or 30 ng, intra-hippocampal	Contextual fear conditioning	Impaired retrieval	[71]
Rat	WIN55,212-2 5 $\mu$ g, intra-amygdala	Inhibitory avoidance	No effect acquisition	[73]
Rat	WIN55,212-2 5 $\mu$ g, intra-hippocampal	Inhibitory avoidance	No effect acquisition	[14]
Rat	$\Delta$ 9-THC 10 mg/kg, IP	Inhibitory avoidance	Impaired acquisition	[67]
Mice	WIN55,212-2 0.25, 0.5 or 1.5 $\mu$ g, intra-hippocampal	Inhibitory avoidance	Impaired retrieval	[72]
Rat	WIN55,212-2 5–50 ng intra-BLA	Inhibitory avoidance	Enhanced consolidation	[56]
Rat	WIN55,212-2 0.3 mg/kg, IP	Object rec- ognition-not habituated	Enhanced retrieval	[56]
Rat	Propofol 300 or 350 mg/kg, IP	Inhibitory avoidance	Enhanced consolidation	[57]

A general summary of the pharmacological studies examining the effects of exogenous cannabinoids on aversive memory paradigms.  $\Delta$ 9-THC:  $\Delta$ 9-tetrahydrocannabinol, *IP* Intraperitoneal

cannabinoids impaired learning and memory with an aversive (water maze, contextual fear conditioning) or non-aversive (object recognition, spatial recognition, social recognition) nature [54, 60]. On the other hand, it was reported that the ECS has a specific role in facilitating fear associated extinction [4, 12, 14–15, 74].

The anandamide uptake inhibitor AM404 [IP: 10 mg/kg; 1  $\mu$ g/ $\mu$ L, intracerebroventricular (ICV)] administered during extinction training facilitated the extinction of startle or freezing elicited by a shock-associated context [53, 75–76]. We found that intra-CA1 WIN55,212-2 facilitated the extinction of inhibitory avoidance whereas intra- basolateral amygdala WIN55,212-2 had no effect on extinction [14, 73].



**Table 2.3** The effects of cannabinoids on extinction

Species	Drug	Test	Effect on extinction	References
Rat	WIN55,212-2 5 µg, intra-CA1	Inhibitory avoidance	Facilitated	[14]
Rat	AM404 200 ng, intra-CA1	Inhibitory avoidance	Facilitated	[14]
Rat	WIN55,212-2 0.25 mg/kg, IP	Contextual fear conditioning	Facilitated	[13]
Rat	WIN55,212-2 0.25 mg/kg, IP	Contextual fear conditioning	Facilitated	[53]
Rat	AM404 10 mg/kg, IP	Contextual fear conditioning	Facilitated	[53]
Rat	AM404 1 µg, ICV	Contextual fear conditioning	Facilitated	[75]
Rat	CBD 2 µg, ICV	Contextual fear conditioning	Facilitated	[75]
Rat	AEA 0.17 ng, intra-CA1	Contextual fear conditioning	Facilitated	[12]
Rat	WIN55,212-2 0.05 µg, intra-IL	Fear-potentiated startle	Facilitated	[74]
Rat	AM404 10 mg/kg, IP	Fear-potentiated startle	Facilitated	[76]
Rat	AM404 0.2 µg, intra-IL	Fear-potentiated startle	Facilitated	[74]
Rat	URB597 0.3 µg, intra-IL	Fear-potentiated startle	Facilitated	[74]
Rat	WIN55,212-2 5 mg/kg, IP	Fear-potentiated startle	No effect	[76]

A general summary of the pharmacological studies examining the effects of exogenous cannabinoids on extinction. *AEA* N-arachidonylethanolamine, *CBD* cannabidiol, *IP* Intraperitoneal, *ICV* intracerebroventricular, *IL* infralimbic

Lin and coworkers have shown that direct infusion of a CB1 receptor agonist, fatty acid amide hydrolase inhibitor, or uptake inhibitor into the ventromedial prefrontal cortex facilitated extinction of a cue-induced fear-potentiated startle response, while infusion of a CB1 receptor antagonist retarded this form of extinction learning [74]. Furthermore, activation of CB1 receptors within this region also reduced startle potentiation in the absence of cue presentation, suggesting that these receptors are not only involved in the extinction of conditioned fear, but also in adaptation to aversive situations in general [74]. Direct microinjection of cannabidiol, a non psychoactive cannabinoid compound, into the prelimbic prefrontal cortex reduced freezing induced by re-exposure to a context previously paired with footshocks [77]. However, in the more ventrally located infralimbic region of the prefrontal cortex, cannabidiol produced an opposite result, increasing the expression of contextual fear conditioning [77]. See Table 2.3 for a general summary of the pharmacological studies examining the effects of exogenous cannabinoids on extinction.

It should be noted that the facilitating effects on extinction were not generalized to another aversively motivated test, the water maze, in which THC did not affect extinction [78]. Furthermore, no effect on extinction was observed in tasks based on appetitive conditioning [79–81].

## **The Interaction Between Stress and Cannabinoids in Their Effects on Emotional Learning**

Although cannabinoid agonists may have different effects on learning and memory, depending on several factors (such as the aversiveness of the task, the memory phase under investigation etc), accumulating data suggest that when cannabinoid agonists are administered in proximity to an environmental stressor, i.e., shortly before or after an exposure to a stressful experience, cannabinoids can normalize the effects of stress on learning and memory [28, 30–32, 73, 82].

We found that the agonist WIN55,212–2 (5 µg) microinjected into the basolateral amygdala had no effect on inhibitory avoidance conditioning or extinction by itself. However, microinjecting WIN55,212–2 into the basolateral amygdala before exposing the rats to an elevated platform stress reversed the enhancing effects of the stressor on inhibitory avoidance conditioning and its impairing effects on extinction [73]. Intra-basolateral amygdala WIN55,212 before elevated platform stress exposure also prevented the stress-induced enhancement of memory consolidation for reduction in reward magnitude [82]. In this negative emotional learning task we measure the decrease in the magnitude of the expected quantity of reinforcements in an alley maze. In contrast to other fear-related negative experiences, reward reduction is more associated with frustration and is assessed by measuring the latency to run the length of the alley to consume the reduced quantity of reward. These findings suggest that cannabinoid receptors in the basolateral amygdala are important modulators of stress-induced modulation of emotional memory [73, 82].

However, when we examined the effects of elevated platform stress on consolidation of memory in a non-emotional object location task, a different picture emerged. Rats were exposed to the elevated platform stress after the acquisition of a non-aversive hippocampal-dependent learning paradigm, the object location task. These rats were exposed to extensive prior habituation to the arena which reduced novelty stress/arousal level. Exposure to the elevated platform stressor impaired consolidation of the location task. The agonist WIN55,212–2 (5 µg) microinjected into the basolateral amygdala did not prevent the stress-induced impairment in consolidation [83].

Taken together, the data strongly points to the integration of endocannabinoids in the stress response and their role in normalizing emotional memory processes, suggesting that the effects of endocannabinoids become evident only in highly aversive situations.

Indeed, using a much more intensive stressor, the single-prolonged stress (SPS) (i.e., restraint for 2 h, forced swim for 20 min, and anesthesia) we found that intra-

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